525 Rec'd PCT/PTO 14 SEP 20

| SUBSTITUTE FORM PTO-1390 | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER 09910-007001 |
|---|---|---|
| **TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371** | | U.S. APPLICATION NO. (IF KNOWN) 09/646204 |
| INTERNATIONAL APPLICATION NO. PCT/FI00/00031 | INTERNATIONAL FILING DATE 18 JANUARY 2000 | PRIORITY DATE CLAIMED 18 JANUARY 1999 |

TITLE OF INVENTION
PURIFICATION PROCESS USING MAGNETIC PARTICLES

APPLICANT(S) FOR DO/EO/US
Helena Seppanen, Pekka Palomaki, Jukka Tuunanen and Timo Karmeniemi

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unsigned)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

*ems 11. to 16. below concern other documents or information included:*

1. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
2. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
3. ☐ A FIRST preliminary amendment.
   ☐ A SECOND or SUBSEQUENT preliminary amendment.
4. ☐ A substitute specification.
5. ☐ A change of power of attorney and/or address letter.
6. ☐ Other items or information:
   ☐
   ☐
   ☐
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   ☐

"Express Mail" mailing label number EL 224698318US
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I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" with sufficient postage on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Samantha Bell

Samantha Bell

430 Rec'd PCT/PTO 14 SEP 2000

| LICATION NO. (IF KNOWN) 09/646204 | INTERNATIONAL APPLICATION NO. PCT/FI00/00031 | ATTORNEY'S DOCKET NUMBER 09910-007001 | |
|---|---|---|---|
| ☒ The following fees are submitted: | | CALCULATIONS | PTO USE ONLY |
| Basic National Fee (37 CFR 1.492(a)(1)-(5)): | | | |
| Search report has been prepared by the EPO or JPO ..... $840 | | $0.00 | |
| nternational preliminary examination fee paid to USPTO (37 CFR 1.482) .. $670 | | $0.00 | |
| No international preliminary examination fee paid to USPTO (37 CFR .482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).... $690 | | $0.00 | |
| Neither international preliminary examination fee (37 CFR 1.482) nor nternational search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $970 | | $970.00 | |
| nternational preliminary examination fee paid to USPTO (37 CFR 1.482) nd all claims satisfied provisions of PCT Article 33(2) to (4) ..... $96 | | $0.00 | |
| ENTER APPROPRIATE BASIC FEE AMOUNT | | $0.00 | |
| urcharge of $130 for furnishing the oath or declaration later than ☐ 20 ☐ 30 nos. from the earliest claimed priority date (37 CFR 1.492(e)). | | $0.00 | |
| Claims | Number Filed | Number Extra | Rate |
| otal Claims | 20 - 20 | 0 | x $18 $0.00 |
| dependent Claims | 3 - 3 | 0 | x $78 $0.00 |
| ultiple Dependent Claims(s) (if applicable) | | | + $260 $260.00 |
| OTAL OF ABOVE CALCULATIONS | | | $1230.00 |
| eduction by ½ for filing by small entity, if applicable. Verified Small Entity atement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28.) | | | $0.00 |
| UBTOTAL | | | $1230.00 |
| ocessing fee of $130 for furnishing the English Translation later than ☐ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(f)) | | | $0.00 |
| OTAL NATIONAL FEE | | | $1230.00 |
| e for recording the enclosed assignment (37 CFR 1.21(h)). The assignment ust be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). | | | $0.00 |
| OTAL FEES ENCLOSED | | | $1230.00 |
| | | | Amount to be refunded |
| | | | Charged |

☒ A check in the amount of $1230.00 to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

OTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

ND ALL CORRESPONDENCE TO:


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30,175
REGISTRATION NUMBER

06612.doc

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Helena Seppanen et al.
Serial No. : 09/646,204
Filed : September 14, 2000
International Application: PCT/FI00/00031
International Filing Date: 18 January 2000
Title : PURIFICATION PROCESS USING MAGNETIC PARTICLES

Art Unit : Unknown
Examiner : Unknown

Commissioner for Patents
Washington, D.C. 20231

## PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the Claims:

In claim 9, line 1, delete "any of claims 1-8" and substitute --claim 1--.

## REMARKS

Claim 9 has been amended for better conformance with the requirements of U.S. practice. We submit that no new matter has been introduced.

Filed herewith under separate cover is an information disclosure statement.

CERTIFICATE OF MAILING BY EXPRESS MAIL

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I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Trademarks, 2900 Crystal Drive, Arlington, VA 22202-3513.

December 21, 2000
Date of Deposit

Victor Makway
Signature

Victor Makway
Typed or Printed Name of Person Signing Certificate

Applicants submit that this application is condition for examination. Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: December 21, 2000

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## Background of the invention

The present invention relates to the purification of biological substances using magnetic particles which bind the material specifically from a mixture. The invention can be used for instance for purifying nucleic acids DNA or RNA.

Magnetic particles can be coated with a separation reagent which reacts specifically with a desired biological substance. The particles and the bound substance are separated from the mixture and thereafter the substance is released from the particles for further prosecution. Nowadays this is done in practice so that the particles are drawn with a magnet against the wall of the vessel containing the mixture, and the liquid is poured or sucked off the vessel. Thereafter a new liquid can be dispensed into the vessel. Manual or automatic equipments for such separation technology are also commercially available (e.g., Spherotec, Inc., AutoMag Processor (USA), Merck Magnetic Rack (Darmstadt, Germany), PerSeptive Biosystems 96 well plate separator, Multi-6 Separator, Solo-Sep Separator (USA), Dynal Magnetic Particles Concentrators).

The old purification technique for DNA involves ultracentrifugation in a dense cesium chloride gradient. However, also magnetic particle technology described above has been used for purifying nucleic acids.

WO 94/18565 (Labsystems Oy) suggests a method and device for magnetic particle specific binding assay, in which magnetic particles are separated from a mixture by a probe comprising a rod movable in a vertical bore and provided with a magnet at the lower end thereof. The probe is pushed into the mixture with the rod in the lower position, whereby the particles are collected on the probe. Then the probe is transferred to another vessel and the rod is pulled in its upper position, whereby the particles are released. Thus all steps of the assay can be carried out in a separate vessel without having to transfer liquids. In the last vessel, a measurement is carried out.

WO 96/12959 (Labsystems Oy) suggests a magnetic particle transfer tool comprising an elongated body with a concavely tapered tip part. The body further comprises means for providing a longitudinal magnetic field to collect particles to the tip of the body. The magnetic field can be eliminated in order to release the

particles. This tool can be used especially for collecting particles from a large volume and releasing them into a very small volume.

## General description of the invention

Now a method according to claim 1 has been invented. Some preferable embodiments of the invention are defined in the other claims.

According to the invention, material to be purified is dispensed in a first medium containing magnetic particles, which have been coated with a binding reagent for the material. The binding reaction takes place, after which the particles are separated by means of a magnetic probe and transferred into a second medium, in which a desired further reaction necessary for the purification may take place. The particles can be transferred similarly via further mediums for carrying out further steps of the purification process. All the vessels may contain the necessary reaction medium ready when the particles are transferred into it. Preferably the particles are also released from the probe in the second and subsequent mediums.

According to the invention, at least one of the mediums contains a surface tension releasing agent. This promotes the complete collection of the particles.

Some of such agents have been used also before in this connection to promote the binding of the substance to be separated, see e.g. Wipat et al., Microbiology (1994), 140, 2067. In these known methods, the particles are not transferred from a vessel to another bu they are held on the wall of the vessel by means of a external magnet, while the medium is removed from the vessel.

The invention can be used especially for purifying nucleic acids, such as ssDNA, dsDNA, and mRNA.

## Brief description of the drawings

The enclosed drawings form a part of the written description.
Figure 1 shows the effect of a detergent in collecting and releasing steps of magnetizable particles.
Figure 2 shows the effect of salt and saccharose in collecting and releasing buffer.
Figure 3 shows the effect of protein in collecting and releasing buffer.
Figure 4 shows the effect of a detergent when magnetic particles of different suppliers were used.

**Detailed description of the invention**

The invention can be used for instance for the purification of cells, viruses, subcellular organelles, proteins, and especially nucleic acid materials.

The magnetic particles are preferably paramagnetic. The size of the particles is usually less than 50 µm, preferably 0.1 - 10 µm, and most preferably 1 - 5 µm. The concentration of the particles may be eg. 0.01 - 5 mg/ml, preferably 0.05 - 3 mg/ml, and most preferably 0.2 - 2 mg/ml.

The particles have been coated or treated with a binding reagent, eg. silicon, lectins and/or other reactive functional groups such a oligonucleotides, antibodies, antigens, streptavidin, or biotin.

The particles are preferably transferred from a vessel to another by using a probe comprising a rod movable in a vertical bore and provided with a magnet at the lower end thereof. The probe is pushed into the mixture with the rod in the lower position, whereby the particles are collected on the probe. Then the probe is transferred to another vessel, and when the rod is pulled to its upper position the particles are released.

Different kind of surface tension releasing compounds, especially water soluble compounds, can be used in the method. Examples of such are:

A. Tensides, such as
- Soaps
- Detergents; including anionic, kationic, non-ionic and zwitterionic compounds

B. Alcohols, such as
- Polyethylene and polyvinyl alcohols and their protein etc. derivatives

C. Proteins

D. Salts and carbohydrates in high concentrations, such as
- NaCl
- Saccharose

Also mixtures of compounds can be used.

Especially tensides such as detergents are suitable. Preferable detergents are ethoxylated anhydrosorbitol esters. The esters may contain eg. about 4 - 20 ethylene oxide groups.

The concentration of a tenside may be eg. 0.001 - 0.5% (w/v), preferably 0.005 - 0.1% (w/v), and most preferably 0.01 - 0.05% (w/v). The concentration of a protein

may be eg. 0.1 - 10% (w/v), preferably 0.25 - 5% (w/v), and most preferably 0.5 - 2% (w/v). The concentraion of a salt may be eg. 0.1 - 10 M, preferably 0.1 - 7 M.

For purification of DNA or mRNA from different sources (for instance, DNA from PCR amplification; DNA from blood, bone marrow or cultured cells; mRNA from eucaryotic total RNA or from crude extracts of animal tissues, cells and plants) the nucleic acids are immobilized by using magnetic particles. The binding can be mediated by the interaction of streptavidin and biotin, whereby particles coated with streptavidin and biotinylated DNA can be used. In addition, DNA can be adsorbed to the surface of the particles. The binding of mRNA can be mediated by Oligo $(dT)_{25}$ covalently coupled to the surface of the particles.

After the immobilization, the nucleic acids are washed several times to remove all the reaction components resulting from the amplification or other contaminants and, e.g., PCR inhibitors.

The washing can be performed by releasing and collecting complexes in a washing buffer and by transfering the complexes to another well containing fresh washing buffer.

For ssDNA purification the immobilized double-stranded DNA can be converted to a single-stranded by incubation with 0.1 M NaOH and using magnetic separation.

For the isolation of mRNA, it can be eluted from the particles by using a low salt buffer.

The purification process can be performed by a magnetic particle processor, in which all the mediums are ready in separate vessel. A surface tension releasing compound is preferably used in each medium. Suitable disposable plates, such as microtitration plates, comprising the necesary vessels can be used. In one plate, several parallel purifications can be accomplished.

**Example of reagents used for a ssDNA purification**

1. Particle suspension in eg. phosphate, Tris or Borate buffered saline, pH 7.4, containing 0.1% BSA, 15 mM $NaN_3$ and eg. 0.02% polyoxyethylene (20) sorbitan monolaurate (Tween 20 ™) as a surface tension releasing agent

2. Binding and Washing buffer (TEN): 10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, eg. 0.02% Tween 20 ™, 15 mM $NaN_3$, pH 7.5

3. TE buffer: 10 mM Tris-HCl, 1 mM EDTA, eg. 0.02% Tween 20 ™, 15 mM $NaN_3$, pH 7.5

4. Melting solution: 0.1 M NaOH, eg. 0.02% Tween 20 ™

5. eg. 0.02% Tween 20 ™ in distilled water, 15 mM $NaN_3$

**Example of reagents used for a mRNA direct purification**

1. Oligo $(dT)_{25}$ particle suspension in PBS, pH 7.4, containing eg. 0.02% Tween 20 ™ and 15 mM $NaN_3$

2. Lysis/binding buffer: 100 mM Tris-HCl, pH 8.0, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM dithiothreitol (DTT), 15 mM $NaN_3$, (eg. 0.02% Tween 20 ™)

3. Washing buffer with LiDS (SDS): 10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS, 15 mM $NaN_3$ (eg. 0.02% Tween 20 ™)

4. Washing buffer: 10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, eg. 0.02% Tween 20 ™, 15 mM $NaN_3$

5. Elution solution: 2 mM EDTA, pH 8.0 , 15 mM $NaN_3$, eg. 0.02% Tween 20 ™

6. Reconditioning solution: 0.1 M NaOH , eg. 0.02% Tween 20 ™

7. Storage buffer Oligo $(dT)_{25}$: 250 mM Tris-Hcl, pH 8, 20 mM EDTA, 0.1% Tween-20, 15 mM $NaN_3$

**Example of the reagents used for a mRNA purification**

1. Binding buffer: 20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 2 mM EDTA, 15 mM $NaN_3$ , eg. 0.02% Tween 20 ™

2. Washing buffer: 10 mM Tris-HCl, pH 8.0, 0.15 mM LiCl, 1 mM EDTA, 15 mM $NaN_3$, eg. 0.02% Tween 20 ™

5. Elution solution: 2 mM EDTA, pH 8.0 , 15 mM $NaN_3$, eg. 0.02% Tween 20 ™

**Example of reagents used for RNA isolation**

1. 4 M guanidium isothiocyanate, 25 mM sodium citrate pH 7.0, 0.5% N-lauryl sarcosine, 0.01 M β-mercaptoethanol

**Example of reagents used for a DNA direct purification**

1. Particle suspension in Lysis buffer (eg. 50 mM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS, 1% 2-mercaptoethanol; 50 mM KCl, 10 - 20 mM Tris-HCl, 2.5 mM $MgCl_2$, pH 8.3, 0.5 Tween 20 ™, 100 µg/ml Proteinase K; 100 mM Tris-HCl, pH 8.5, 5 mM EDTA, 1% SDS, 500 µg/ml Proteinase K) containing 15 mM $NaN_3$

2. Washing buffer containing 15 mM $NaN_3$ and eg. 0.02% Tween 20 ™

3. Resuspension buffer containing 15 mM $NaN_3$ and eg. 0.02% Tween 20 ™

**Example of the purification process of PCR products by a magnetic particle processor at room temperature**

The reagents are dispensed into a subsequent wells of a plate.

Example of a reagent configuration:

Well 1. Sample (biotinylated DNA, PCR amplicons)
Well 2. Streptavidin coated magnetic particles in washing buffer
Wells 3 - 5. Washing buffer
Well 6. NaOH
Well 7. TE buffer
Well 8. Distilled water

Example of processing steps:

Well 2. Mixing, washing and collecting of particles, moving of them into well 3
Well 3. Washing of particles, moving of them into well 4
Well 4. Washing of particles, moving of them into well 1
Well 1. Sample incubation 10', moving of particles into well 4
Well 4. Washing of particles, moving of them into well 5
Well 5. Washing of particles, moving of them into well 6
Well 6. Incubation 5' in melting solution, moving of particles into well 4
Well 4. Washing of particles, moving of them into well 5
Well 5. Washing of particles, moving of them into well 7
Well 7. Rinsing of particles, moving of them into well 8
Well 8. Releasing of particles

**The effect of surface tension releasing agent (STRA) in collecting and releasing steps of magnetizable particles**

Streptavidin coated magnetic particles (sizes: Scigen streptavidin 3 µm; Scigen; SPHERO™ streptavidin 4 - 4.5µm, Spherotec, Dynabeads M-280 streptavidin 2.8 µm, Dynal) were saturated with biotinylated alkaline phosphatase (Calbiochem, San Diego, CA) for 1 h at +37 °C. Saturated particles were first washed to remove the unbound alkaline phosphatase and were then used to examine the effect of STRA in collecting and releasing steps of a magnetic particle processor. The instrument settings of these examples were adjusted from 20 µl to 200 µl and the capacity range of the processor was 1 - 24 samples per run. The processor utilized a rod magnet (cylindrical NdFeB axially magnetized, length 2 mm, width 3 mm) in polypropene tube (outer width 4.5 mm).

Briefly, the particles were processed by releasing and collecting them from well to well so that the whole process comprised of 10 steps. The amount of particles, which remained into the wells after the collection, was estimated with alkaline phosphatase assay. Samples (10 µl) from each well were transferred to an empty microtitration plate (round-bottomed wells, width 6.5 mm).

In this assay alkaline phosphatase saturated particles (0.016 µg - 1 µg particles / 10 µl diluent) were used as standards. Into the wells containing 10 µl samples and standards were added 100 µl pNPP-substrate diluted in diethanolamine (DEA) buffer (Labsystems). The substrate was incubated for 15 minutes at +37 °C with continuous shaking (900 rpm) in Labsystems iEMS Incubator/Shaker. The reaction was stopped by adding 100 µl 1M NaOH into each well and the absorbances at 405 nm were measured by photometer (Labsystems Multiskan).

The amount of remaining particles was determined from a linear standard curve and finally results were expressed as percentage of initial amount of particles (0.2 mg/well).

In Fig 1. is shown the effect of detergent (Tween 20 ™) in different concentrations. The degree of remaining particles (Scigen streptavidin) were over 3% / well, when surface tension releasing agent was not added into the collecting and releasing buffer. When the detergent concentration was ≥ 0.00125%, the particles were collected efficiently.

In Fig 2. is shown the effect of salt and saccharose in collecting and releasing buffer. By adding these components into the buffer, the collection of particles (Scigen streptavidin) was more efficient.

In Fig 3. is shown the effect of a protein (casein) which was improving the collecting steps of particles (SPHERO™ streptavidin) in some degree.

In Fig. 4 is shown the effect of detergent (Tween 20 ™) when the magnetic particles of different suppliers were used.

## Claims

1. A process for the purification of a substance, wherein
- material containing the substance, and magnetic particles coated or treated with a reagent which binds the particles to the substance are dispensed in a first medium,
- a binding reaction is let to take place, in which reaction the substance is bound to the particles, and
- a magnetic probe is pushed into the medium, whereby the particles adhere to the probe, and the probe together with the particles and the substance bound to them is transferred to a second medium, and if desired, separated from the second medium and transferred to a third medium,

characterized in that
- a surface tension releasing agent is dispensed at least on of the mediums, preferably at least to the first medium, and most preferably to all mediums, before the probe and the particles are transferred from it.

2. A method according to claim 1, wherein the surface releasing compound is a tenside, alcohol, protein, or a salt or carbohydrate.

3. A method according to claim 1 or 2, wherein the surface tension releasing compound is a tenside, such as a detergent.

4. A method according to claim 3, wherein the concentration of the tenside is 0.001 - 0.5% (w/v), preferably 0.005 - 0.1% (w/v), and most preferably 0.01 - 0.05% (w/v).

5. A method according to claim 1 or 2, wherein the surface tension releasing compound is a protein.

6. A method according to claim 5, wherein the concentration of the protein is 0.1 - 10% (w/v), preferably 0.25 - 5% (w/v), and most preferably 0.5 - 2% (w/v).

7. A method according to claim 1 or 2, wherein the surface tension releasing compound is a salt.

8. A method according to claim 7, wherein the concentration of the salt is 0.1 - 10 M, preferably 0.1 - 7 M.

9. A method according to any of claims 1-8 for the prurification of cells, viruses, subcellular organelles, proteins, or nucleic acid materials.

10. A method according to claim 9 for the purification of nucleic acid materials.

11. A method according to any of claims 1-10, wherein the size of the magnetic particles is less than 50 μm, preferably 0.1 - 10 μm, and most preferably 1 - 5 μm.

12. A method according to any of claims 1-11, wherein the concentration of the magnetic particles is 0.01 - 5 mg/ml, preferably 0.05 - 3 mg/ml, and most preferably 0.2 - 2 mg/ml.

13. A method for separating magnetic particles by means of a magnetic probe from a medium, characterized in that a surface tension releasing agent is dispensed into the medium before the particles are separated.

14. A method for improving the adherence of magnetic particles from a liquid medium to a magnetic probe to be pushed into the medium, characterized in that a surface tension releasing agent is dispensed into the medium before the particles are adherted to the probe.

09/646204
WO 00/42432 PCT/FI00/00031



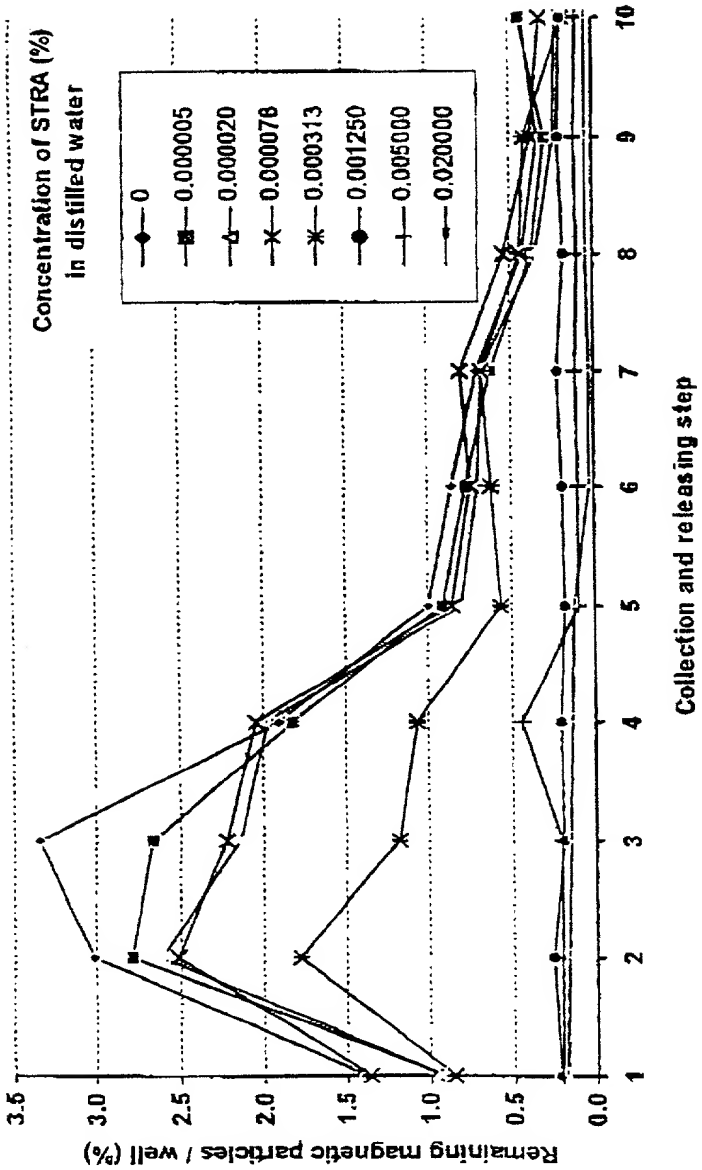


Fig. 1

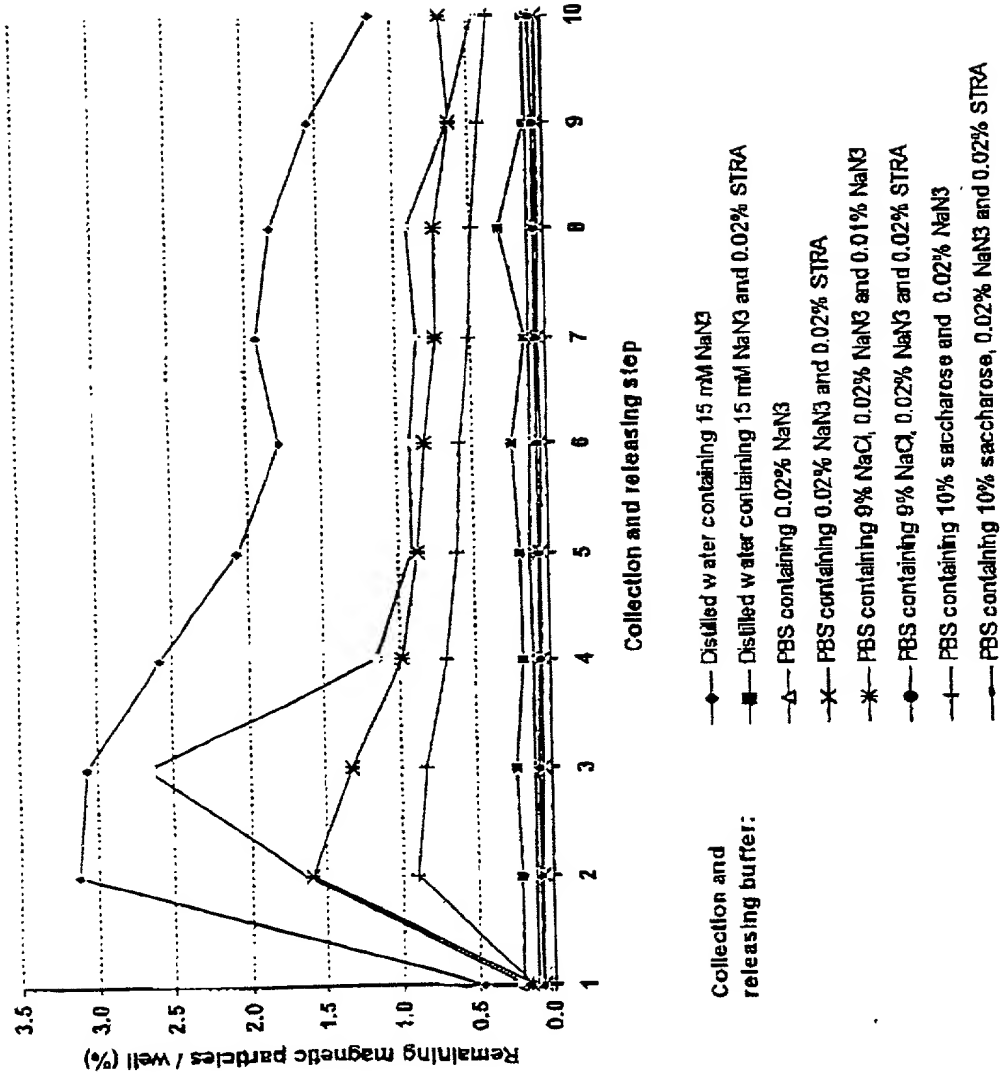
Remaining magnetic particles / well (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step
Collection and releasing buffer:
Distilled water containing 15 mM NaN3
Distilled water containing 15 mM NaN3 and 0.02% STRA
PBS containing 0.02% NaN3
PBS containing 0.02% NaN3 and 0.02% STRA
PBS containing 9% NaCl, 0.02% NaN3 and 0.01% NaN3
PBS containing 9% NaCl, 0.02% NaN3 and 0.02% STRA
PBS containing 10% saccharose and 0.02% NaN3
PBS containing 10% saccharose, 0.02% NaN3 and 0.02% STRA

Fig. 2

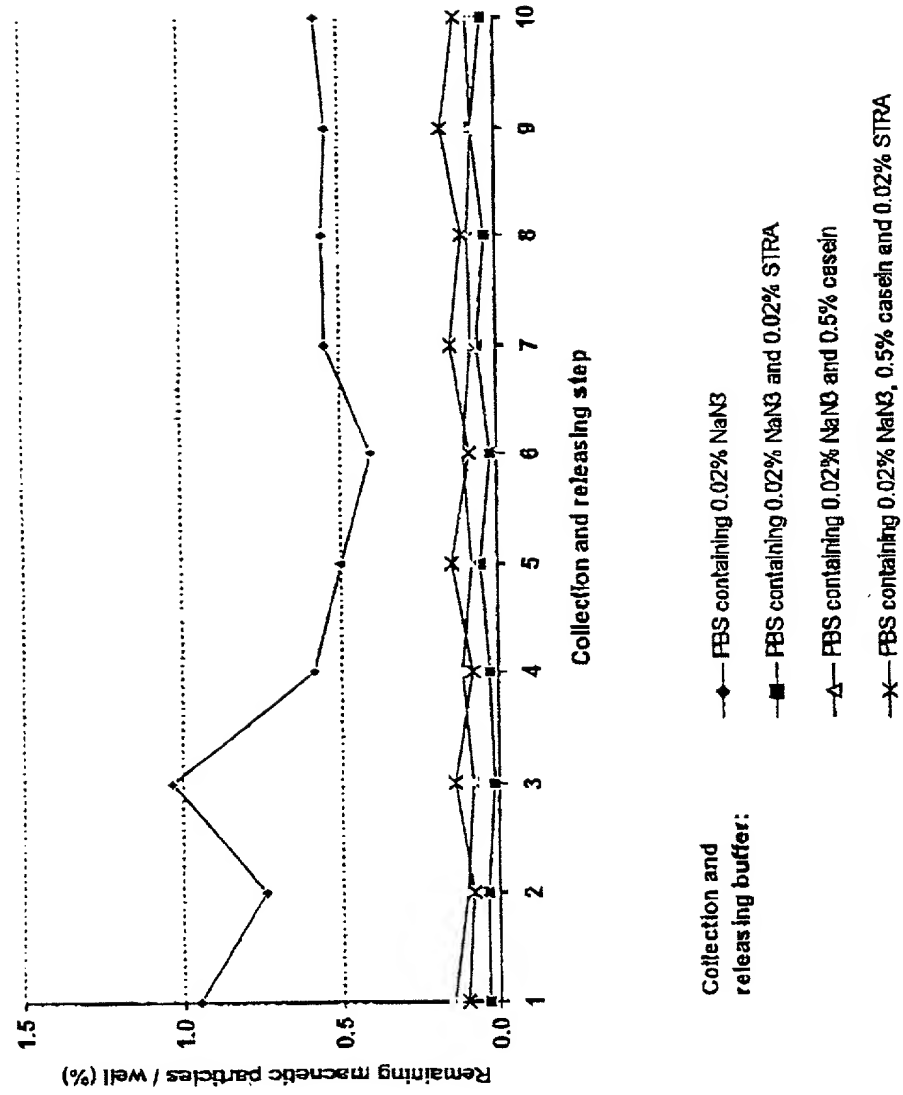
Remaining magnetic particles / well (%)
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step
Collection and releasing buffer:
PBS containing 0.02% NaN3
PBS containing 0.02% NaN3 and 0.02% STRA
PBS containing 0.02% NaN3 and 0.5% casein
PBS containing 0.02% NaN3, 0.5% casein and 0.02% STRA

Fig. 3

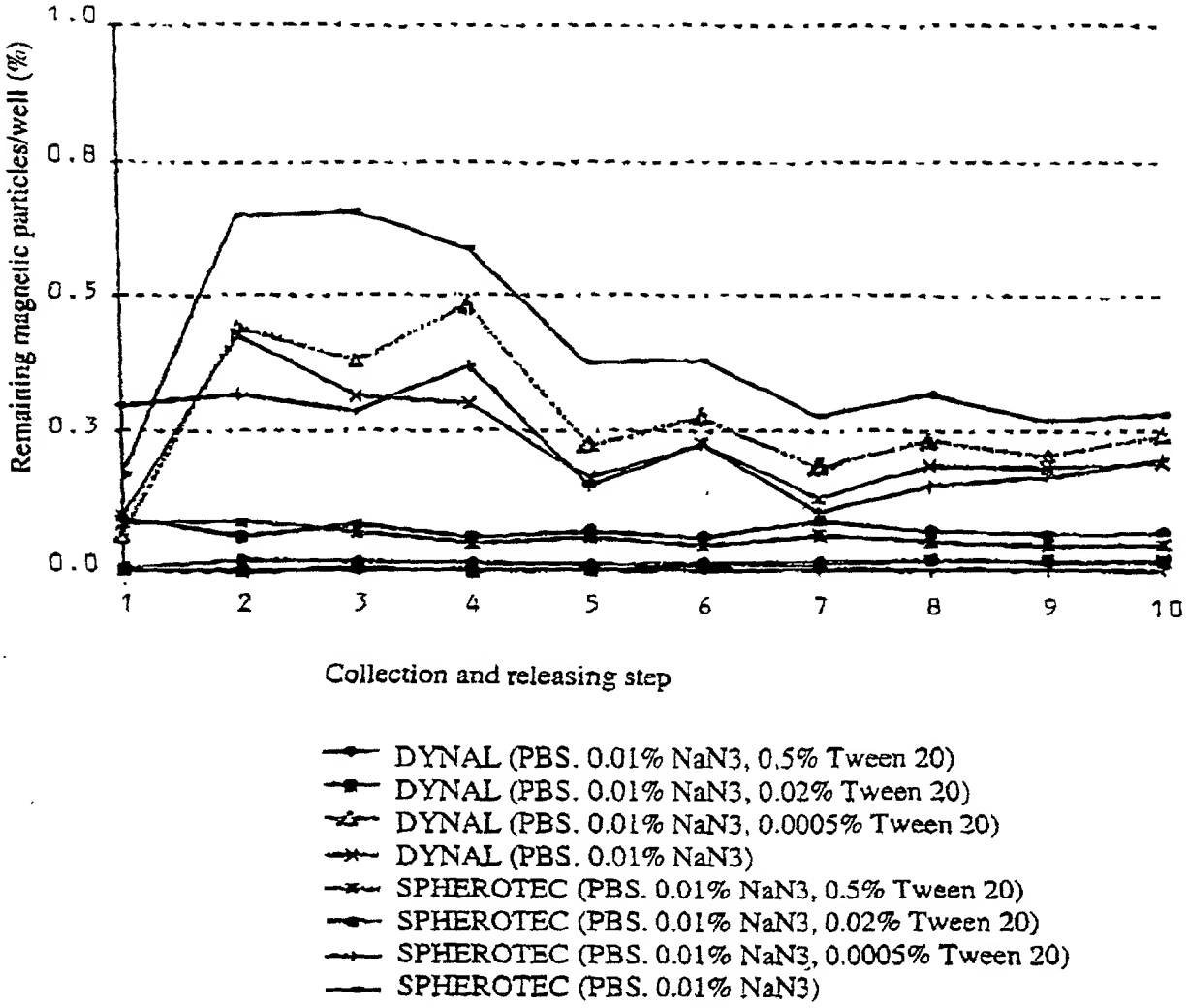
Remaining magnetic particles/well (%)
1.0
0.8
0.5
0.3
0.0
1
2
3
4
5
6
7
8
9
10
Collection and releasing step
DYNAL (PBS. 0.01% NaN3, 0.5% Tween 20)
DYNAL (PBS. 0.01% NaN3, 0.02% Tween 20)
DYNAL (PBS. 0.01% NaN3, 0.0005% Tween 20)
DYNAL (PBS. 0.01% NaN3)
SPHEROTEC (PBS. 0.01% NaN3, 0.5% Tween 20)
SPHEROTEC (PBS. 0.01% NaN3, 0.02% Tween 20)
SPHEROTEC (PBS. 0.01% NaN3, 0.0005% Tween 20)
SPHEROTEC (PBS. 0.01% NaN3)

Fig. 4

Attorney's Docket No.: 09910-007001
Client's Ref. No.: BP100140/EH/TUK

# COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled PURIFICATION PROCESS USING MAGNETIC PARTICLES, the specification of which:

[] is attached hereto.
[] was filed on _ as Application Serial No. _ and was amended on _____.
[X] was described and claimed in PCT International Application No. PCT/FI00/00031 filed on 18 January 2000 and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

| U.S. Serial No. | Filing Date | Status |
|---|---|---|

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

| U.S. Serial No. | Filing Date | Status |
|---|---|---|

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

| Country | Application No. | Filing Date | Priority Claimed |
|---|---|---|---|
| Finland | 990082 | 18 January 1999 | [X] Yes [] No |
| | | | [] Yes [] No |

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

1-00

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